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Effect of Valproic Acid on Maternal - Fetal Heart Rates and Coupling in Mice on Embryonic day 15.5 (E15.5)

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***Abstract*— Prenatal uptake of valproic acid (VPA) was associated with increased risk of fetal cardiac anomalies and autism spectrum disorder (ASD), but uptake of VPA is considered the only effective treatment for epilepsy and other neurological disorders. Up until now, little is known about the effect of VPA on maternal – fetal heart rate (HR) coupling patterns; therefore, this study aims at studying such patterns in mice on embryonic day 15.5 (E15.5). At E12.5, 8 mothers were injected with VPA (VPA group) and another 8 mothers were injected with saline (control group). At E15.5, electrocardiogram (ECG) records of 15 minutes were collected from the 16 mothers and 25 fetuses. A maximum of 5-minutes and a minimum of 1-minute were selected from the ECG data for analysis. Mean RR intervals and coupling ratios and their occurrence percentages were calculated per 1-minute. 1-minute analysis was done for periods with no arrhythmia and clear R peaks. The total number of 1-minute segments that were analyzed was 56 for the saline group and 54 for the VPA group. The correlation analysis between the 1:3 and 2:6 coupling ratios and RR intervals revealed that the ratios were significantly correlated in the saline group, whereas no significant correlations were observed in the VPA group. The results further revealed that fetal RR intervals are strongly correlated with maternal RR intervals in the saline group, but the same correlation is different in the VPA group. The presented results imply that maintaining certain coupling patterns are important for proper fetal cardiac development and maternal uptake of VPA may affect maternal-fetal HRs interactions.**

I. INTRODUCTION

Mice and humans share similar cardiac anatomy, however, compared to humans, a mouse heart develops relatively fast, and it takes less than three weeks for a fetal mouse to fully develop [1]–[3]. The fast development of cardiac mouse makes genetically modified mouse models optimal to understand the factors involved in fetal cardiac development and maternal–fetal cardiac coupling interactions.

The mechanisms involved in the coupling interactions are still not known and more research is needed to understand how maternal heart rate (HR) patterns affect that of the fetus. Previous studies have shown that maternal mice injected with propranolol [2], [4] and atropine [5] had maternal-fetal coupling interaction patterns different than the control group. The latter studies imply that maternal uptake of certain drugs may eventually affect fetal HRs, hence, it is important to study such effects using mice models for the development of advanced diagnostic tools.

Valproic acid (VPA) is a drug used for treating bipolar disorders, epilepsy, and neurological disorders [6], [7]. Nevertheless, VPA was associated with increased risk of fetal cardiac anomalies [7], limb defects [7] and autism spectrum disorder (ASD) [6], [8]. Despite the risks involved in prenatal uptake of VPA, it is considered to be the only effective treatment for epilepsy [6]. So far, the pathological pathways involved in VPA are not thoroughly understood yet, especially in the fetal development, therefore, more research is needed to understand how VPA can impact fetal development [8]. A study by *Y. Kasahara et al.* [8] showed that maternal mice injected with VPA at E12.5 impacted fetal development by analyzing their electrocardiogram (ECG) records. Analysis of HRs collected from fetal mice showed that the fetal short-term variability (STV) in VPA mice was significantly different than the same of mice treated with saline [8]. However, it is still unknown how maternal intake of VPA can affect maternal-fetal HRs coupling interactions in mice. Therefore, the aim of this study is to investigate the impact of VPA on coupling interactions. In addition, the effect of coupling parameters on fetal and maternal RR intervals will also be explored.

II. METHODS

A. Mice and experimental protocol

The study protocols of this study were approved by the Tohoku University Committee for Safety Management of Animals (Sendai, Japan); the study's approval number is 2017MdA-334. C57BL6/J female mice (7-19 weeks of age) were housed with 3-5 male mice of similar age in cages under control lightning of 12h:12h light-dark cycle. Mice had unlimited access to water and food. On the embryonic day 12.5 (E12.5), 600 mg/Kg of VPA sodium salt (VPA; Sigma, St. Louis, MO, USA) dissolved in saline solution was injected to the subcutaneous fat of the pregnant mother's neck (VPA mice) [8]. The control group of mice had only saline solution injected to them at the same location. Maternal ECG (MECG) and fetal ECG (fECG) were collected from the female mice on

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E15.5. The ECG recording system set up is explained in detail in our previous study [9]. Before taking ECG measurements, pregnant mice were anesthetized with subcutaneous ketamine (Ketalar 500 mg, 100 mg/kg, Daiichi-Sankyo), xylazine (Rompun Inj Solution 2%, 10mg/kg, Bayer) and inhalational isoflurane (0.5%, 260 ml/min; Forane AbbVie Inc.) [8]. ECG recordings were carried for 15 minutes at a sampling rate of 1000 Hz. In this study, ECG data that were collected from 8 VPA mice and 8 saline mice were used. fECG data was collected from 2 fetuses per mother and due to noise, only 12 saline fECG data and 13 VPA fECG data were used for analysis.

B. ECG analysis

R peaks in fECG and MECG were automatically detected using a code written in MATLAB. After that, average RR intervals, standard deviation (std) and root mean square of the successive differences (RMSSD) were calculated per 1-minute. 1-minute segments were selected after the first 20 seconds of recording and before the 10^{th} minute to avoid beats with arrhythmia. For each mouse, a maximum of 5-minutes and a minimum of 1-minute were selected for analysis. The analysis was done per 1-minute and the total number of 1-minute segments that was analyzed for the saline group was 56 and the same was 54 for the VPA group. 1-minute analysis was done for periods that had clear R peaks only.

C. Coupling analysis

Coupling analysis was performed by counting the number of maternal beats occurring per one and two fetal beats. A one fetal beat was considered to be the interval located between the first fetal R peak and the second fetal R peak. Two fetal beats were considered to be the interval between the first fetal R peak and the third fetal R peak.

A maternal beat was counted when it was located in the interval $t_1 \leq t < t_2$, where t_1 indicates the occurrence of the first fetal R peak and t_2 indicates the occurrence of the second or third fetal R peak. The number of maternal beats per 1 and 2 fetal beats were counted to see the coupling ratio combinations found in mice in each 1-minute segment.

After counting the number of maternal beats per 1 or 2 fetal beats, a coupling occurrence percentage was calculated per 1-minute. Fig. 1 shows an example of coupling occurrence percentage calculation per 1 fetal beat for an interval of 800 ms; the ECG tracings belong to a saline mouse. The occurance percentages were calculated for the 56 1-minute segments of saline and the 54 1-minute segments of the VPA group. The occurance percentages of both groups were then compared by using frequency distribution. For further comparison between both groups, the correlation coefficient of the relationship between the following pairs of variables were calculated: fetal RR and occurance percentages, maternal RR and occurance percentages, fetal RR and maternal RR.

III. Results

The study investigated the effect of VPA on the HRs and coupling interactions. Table I shows a comparison in RR interval and RMMSD between the saline and VPA groups. Table 1 shows that the maternal and fetal HR parameters are less than the same of VPA group. The frequency of occurrence of coupling ratios that occurred per 1 and 2 fetal beats is shown in Fig. 2. Fig. 2 shows that the VPA group has coupling ratios that did not exist in the saline group such as 1:1, 1:6, 2:2, and 2:3. Furthermore, the frequency of occurrence of coupling ratio of 1:3 and 2:6 is noticeably lower in the VPA group.

TABLE I
Mean RR interval and RMSSD of FECG and MECG of the Saline and VPA group.

Feature	**Saline (n=56)**	**VPA (n=54)**
Mean fetal RR ± std (ms)	705 ± 126	786 ± 136
Fetal RMSSD (ms)	10 ± 13	25 ± 22
Mean maternal RR (ms) ± std (ms)	234 ± 23	275 ± 26
Maternal RMSSD (ms)	10 ± 5	17 ± 17

Since coupling ratios 1:3 and 2:6 were noticeably different between both treatment groups, they were used in the correlation analysis in Table II. Table II shows that the correlation patterns in saline group is different than the VPA group. For further comparison between the saline and VPA group, the correlation between the fetal RR intervals and maternal RR intervals was investigated and the results are shown in Fig. 3.

TABLE II
Correlation analysis between RR intervals and the 1:3 and 2:6 coupling ratios for each treatment group.

Feature	**Coupling Ratio [Fetal beat: Maternal beat]**	
	1:2	1:3
Saline fetal RR	*0.39**	0.11
VPA fetal RR	0.16	0.19
Saline maternal RR	*0.58**	*0.36**
VPA maternal RR	-0.14	-0.26

* $P < 0.05$, correlation coefficient significance.

IV. Discussion

In this study, coupling patterns were compared between the saline (control) and VPA group. Coupling or R peak pairing patterns were evaluated by calculating the changes in fetal HRs to maternal HRs in 1-minute.The results of this study showed that the coupling patterns in the VPA group was different compared to the saline group because VPA caused an increase in both maternal and fetal RR intervals as shown in Table I. Based on the results in Fig. 2, it is revealed that VPA lowered the occurrence frequency of the 1:3 and 2:6 coupling ratios which proved to be significant for maintaining normal RR intervals as shown in Table II. The significant correlation

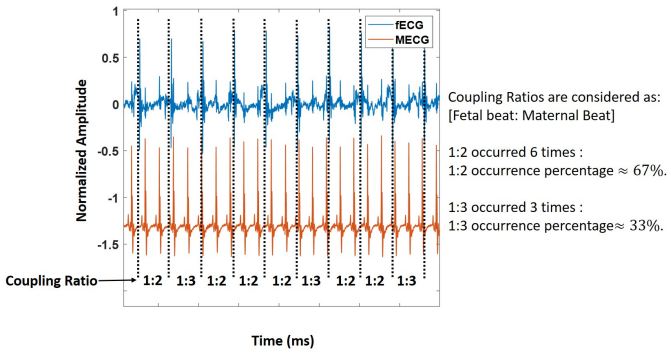

Fig. 1. An example of coupling occurrence percentage calculation per 1 fetal beat for 800 ms. The number of R maternal peaks occurring between two fetal R peaks were counted to determine a coupling ratio. In this example, occurrence percentages were calculated for 1:2 and 1:3 since they are the only coupling ratios that were found in the 800 ms period. The ECG tracings belong to a saline mouse.

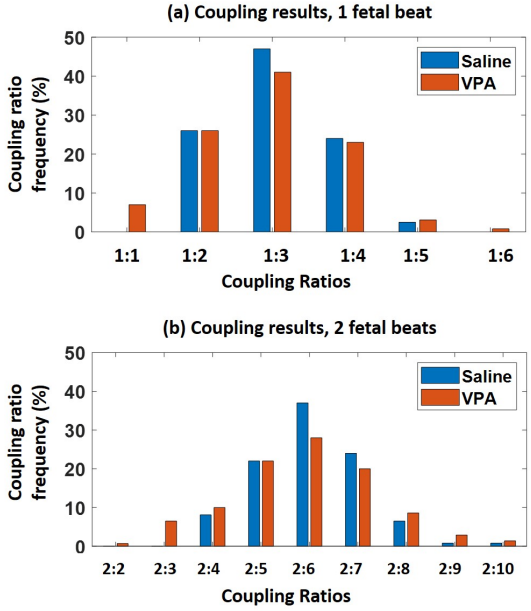

Fig. 2. Frequency of coupling ratio occurrences per 1 (a) and 2 (b) fetal beats. VPA and saline groups are noticeably different at 1:3 and 2:6 coupling ratios.

between coupling ratios and RR intervals prove that maternal HRs are important for the regulations of fetal HRs. The latter is further confirmed in Fig. 3 in which it is shown that under normal conditions, maternal RR intervals are positively correlated with fetal RR intervals. The strong correlation coefficient ($r = 0.75$) proves that fetal heart is strongly affected by maternal conditions. Maternal mice that were injected with VPA had a disturbed fetal and maternal RR relationships as shown in Figure 3b. Because the correlation between maternal RR interval and fetal RR interval changed due to VPA, the coupling ratios were affected as well.

The results in Table II implies that for a healthy fetal mouse development, it is important to maintain a

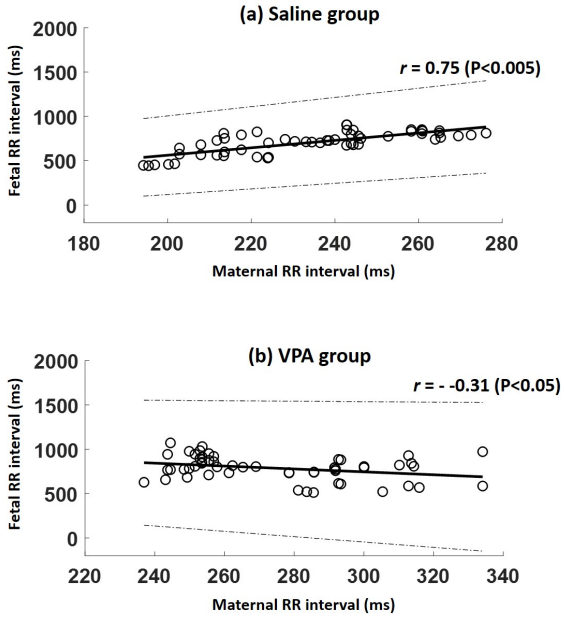

Fig. 3. Comparison between saline and VPA in terms of fetal RR and maternal RR interval correlations. (a) Maternal RR intervals are strongly positively correlated with fetal RR intervals in saline. (b) Maternal RR intervals are negatively correlated with fetal RR intervals in VPA.

coupling ratio of 1:3 and 2:6 at E15.5. Although the saline mice had different HRs, they still maintained 1:3 and 2:6 coupling ratios most of the time. Coupling ratios are indicative of fetal RR intervals to maternal RR intervals ratios, in other words, a 1:3 coupling ratio indicates that a maternal RR interval is 3 times shorter than a fetal RR interval. Because VPA affected fetal and maternal RR interval values, it ended up affecting the coupling ratios. By looking at Fig. 2, it is shown that the VPA group had unique coupling ratios that are absent in the control group such as 1:1, 1:6, 2:2 and 2:3. The latter coupling ratios affected the rate of occurrence of the 1:3 and 2:6 ratios which were the most occurring coupling ratios in both treatment groups. The changes in HR patterns between the saline and VPA groups are attributed to the changes in the sympathetic and parasympathetic activity [8]. The sympathetic and parasympathetic systems are critical for the regulation of HRs, and because of VPA, such regulations could be disturbed [8]. In [8], VPA was shown to affect fetal STV and after birth, fetuses which were exposed to VPA had different heart rate variability (HRV) patterns. The latter findings indicate that VPA affects the activity of the fetal central nervous system [8].

Studying coupling patterns could help in developing new techqnies for prenatal HR analysis because coupling takes into consideration both maternal and fetal factors. Therefore, coupling analysis could help in investigating the changes occuring in fetal HRs due to abnormal maternal conditions such as arrhythmia. The work in this study focused on E15.5 only and similar analysis

should be repeated for other gestational ages (GAs) as well since the HRs patterns change significantly with GA. Understanding maternal – fetal coupling interactions is not only important for diagnostic purposes; it could be also used to develop models to estimate fetal age as was previously done by *A. Khandoker et al.* [10] in which it is addressed that coupling ratios are important parameters for estimating fetal age.

V. CONCLUSIONS

The results of this study showed that maternal uptake of VPA can affect maternal-fetal coupling interactions. Maternal-fetal coupling interactions are important for the regulations and maintenance of normal fetal HRs. The study focused on E15.5, thus, more research should be done at different gestational age to investigate how maternal-fetal coupling ratios change with GA.

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